



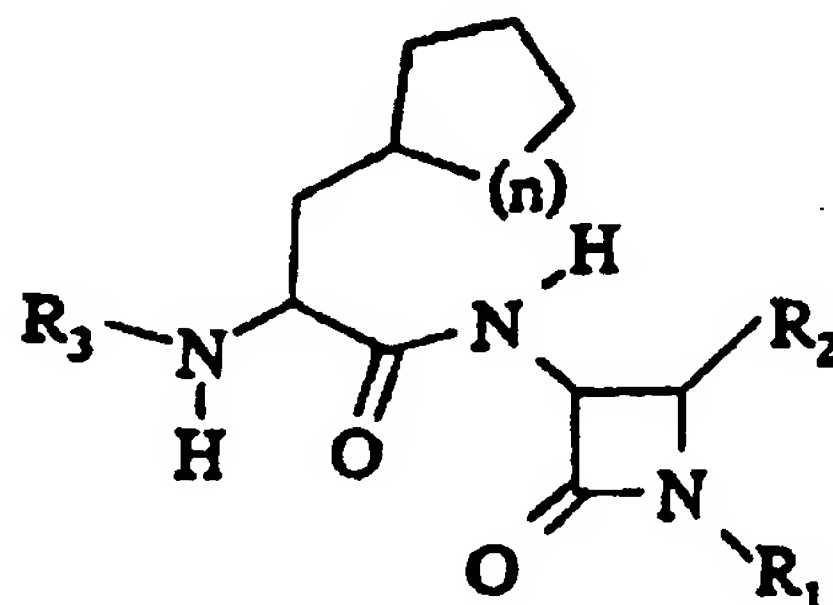
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(51) Int.Cl.<sup>6</sup> C07K 5/06, A61K 38/05

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(54) **DERIVES DE 3-(2-AMINO-2-CYCLOALKYLE METHYLE)-  
ACETAMIDO AZETIDINE-2-ONE SUBSTITUEE EN 4  
REGULATEURS DE CYSTEINE PROTEINASE**

(54) **4-SUBSTITUTED-3-(2-AMINO-2-CYCLOALKYL METHYL)-  
ACETAMIDO AZETIDIN-2-ONE DERIVATIVES AS  
CYSTEINE PROTEINASE REGULATORS**



(I)

(57) Dérivés de 3-(2-amino-2-cycloalkyle méthyle)-acétamido azétidine-2-one substituée en 4 de la formule (I) et leurs sels. Dans ladite formule, n est égal à 1, 2 ou 3; R<sub>1</sub>, R<sub>2</sub> et R<sub>3</sub> sont tels que définis. Les produits en question offrent une excellente inhibition de cystéine protéinase, et on peut les utiliser pour le traitement de différentes maladies, à savoir par exemple: myopathie musculaire progressive, infarctus du myocarde, résorption osseuse, arthrite, métastases cancéreuses, emphysème pulmonaire, choc septique, ischémie cérébrale, fonction mnésique, maladie d'Alzheimer et cataracte, paludisme, dégradation de la membrane basale des capillaires du glomérule rénal, infection bactérienne, maladies inflammatoires, maladies parasitaires et infections virales.

(57) In accordance with the present invention, there are provided 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula (I), wherein n is 1, 2 or 3; in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are as defined herein, and salts thereof, which exhibit excellent cysteine proteinase inhibitory activity and which can be used for treatment of different diseases such as muscular dystrophy, myocardial infarction, bone resorption, arthritis, cancer metastasis, pulmonary emphysema, septic shock, cerebral ischemia, memory function, Alzheimer and cataract, malaria, glomerular basement membrane degradation, bacterial infection, inflammatory diseases, parasitic infections, and viral infections.

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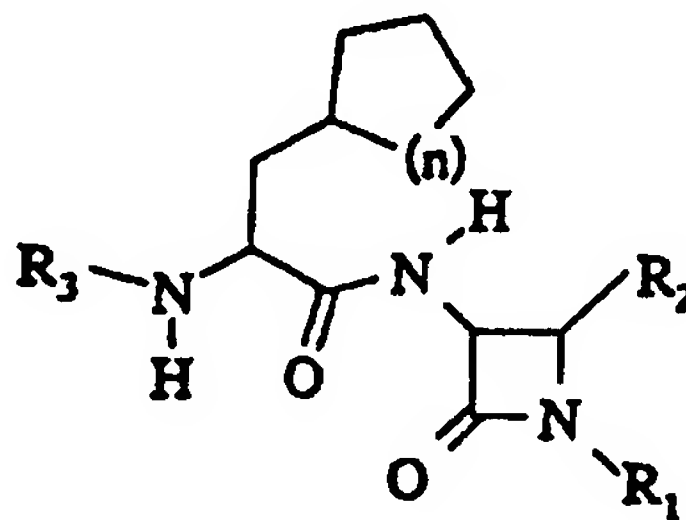
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TEINE PROTEINASE REGULATORS

## (57) Abstract

In accordance with the present invention, there are provided 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula (I), wherein n is 1, 2 or 3; in which R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are as defined herein, and salts thereof, which exhibit excellent cysteine proteinase inhibitory activity and which can be used for treatment of different diseases such as muscular dystrophy, myocardial



(I)

infarction, bone resorption, arthritis, cancer metastasis, pulmonary emphysema, septic shock, cerebral ischemia, memory function, Alzheimer and cataract, malaria, glomerular basement membrane degradation, bacterial infection, inflammatory diseases, parasitic infections, and viral infections.

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4-SUBSTITUTED-3-(2-AMINO-2-CYCLOALKYL METHYL)- ACETAMIDO  
AZETIDIN-2-ONE DERIVATIVES AS  
CYSTEINE PROTEINASE REGULATORS

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This application claims priority of United States Provisional patent  
5 application Serial Number 60/026,514, filed September 23, 1996.

Background of the Invention

Cysteine proteinases containing a highly reactive cysteine residue with  
a free thiol group at the active site have been known as playing an important  
role in certain conditions distinguished by aberrant protein turnover such as:  
10 muscular dystrophy (Am. J. Pathol. 1986, 122, 193-198; Am. J. Pathol.  
1987, 127, 461-466), myocardial infarction (J. Am. Coll. Cardiol. 1983, 2,  
681-688), bone resorption (Biochem. J. 1991, 279, 167-274; J. Biol. Chem.  
1996, 271, 2126-2132; and Biochem. Biophys. Acta 1992, 1116, 57-66),  
arthritis (Arthritis Rheumatism 1994, 37, 236-247; and Biochem. Pharmacol.  
15 1992, 44, 1201-1207), cancer metastasis (Cancer Metastasis Rev. 1990, 9,  
333-352), pulmonary emphysema (Am. Rev. Respir. Dis. 1975, 111, 579-586),  
septic shock (Immunol. Today 1991, 11, 404-410, Biochemistry 1994, 33,  
3934-3940), cerebral ischemia, memory function, Alzheimer and cataract  
(TIPS 1994, 15, 412-419, Bioorg. Med. Chem. Lett. 1995, 4, 387-392, Proc.  
20 Natl. Acad. Sci. USA 1991, 88, 10998-11002), malaria (J. Med. Chem. 1995,  
38, 5031-5037), glomerular basement membrane degradation (Biochem.  
Bioph. Acta 1989, 990, 246-251), bacterial infection (Nature 1989, 337, 385-  
386), inflammatory diseases (Protein Science 1995, 4, 3-12), parasitic  
infections (Annu. Rev. Microbiol. 1993, 47, 821-853; Parasitol. Today 1990,  
25 6, 270-275), and viral infections (Biochem. 1992, 31, 7862-7869).

A variety of cysteine proteinases have been shown to be present in  
mammalian tissue. The most notable of these proteinases are the lysosomal  
cathepsins (cathepsin B, H, S, L and K) and the cytoplasmic Ca<sup>+2</sup> dependent  
enzymes, the calpains. These enzymes are, therefore, excellent targets for  
30 specific inhibitors as therapeutic agents for the conditions such as those

noted above.

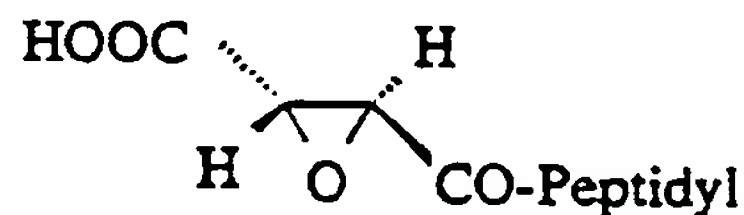
Cysteine proteinases are inhibited by several types of peptide derived inhibitors such as peptidyl aldehyde (Eur. J. Biochem. 1982, 129, 33-41), chloromethyl ketone (Acta. Biol. Med. Ger. 1981, 40, 1503-1511), diazomethyl ketone (Biochemistry 1977, 16, 5857-5861), monofluoromethyl ketone (Biochemical Pharmacology 1992 44, 1201-1207), acyloxy methyl ketone (J. Med. Chem. 1994, 37, 1833-1840), O-acyl hydroxamates (Biochem. Biophys. Research Communications 1988, 155, 1201-1206), methyl sulphonium salts (J. Biol. Chem. 1988, 263, 2768-2772) and epoxy succinyl derivatives (Agric. Biol. Chem. 1978, 42, 523-527) without significantly inhibiting other classes of proteinases.

#### Summary of the Invention

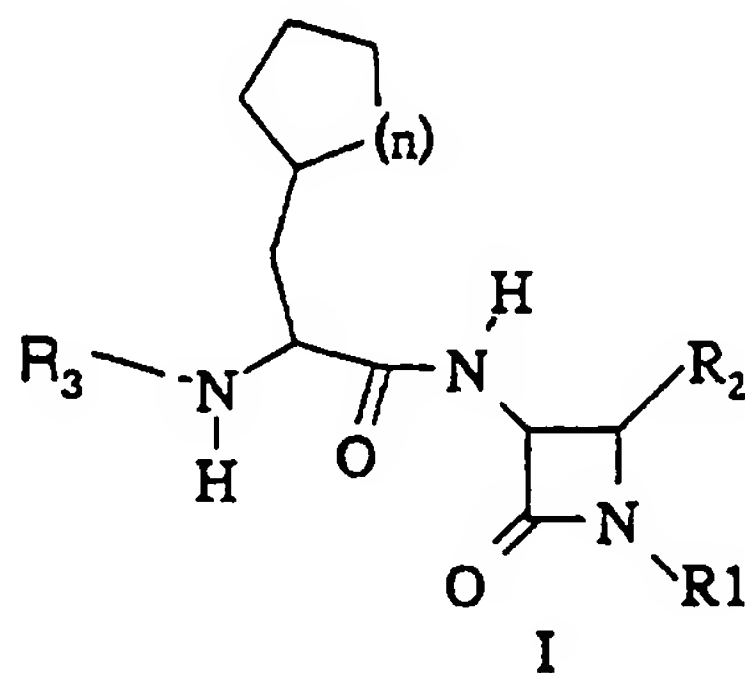
Our laboratory has been extensively involved in search for novel cysteine proteinase regulators and found that 4-substituted-3-(amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives exhibit excellent activity and selectivity within the class of cysteine proteinases. There is an ongoing need to improve *in vivo* efficacy by improving plasma stability and pharmacokinetics.

#### Peptidyl-CO-Y

Y = H, CH<sub>2</sub>Cl, CHN<sub>2</sub>, CH<sub>2</sub>F,  
CH<sub>2</sub>OCOAr, NHOCOR,  
CH<sub>2</sub>S-(CH<sub>3</sub>)<sub>2</sub>



Epoxysuccinyl derivative

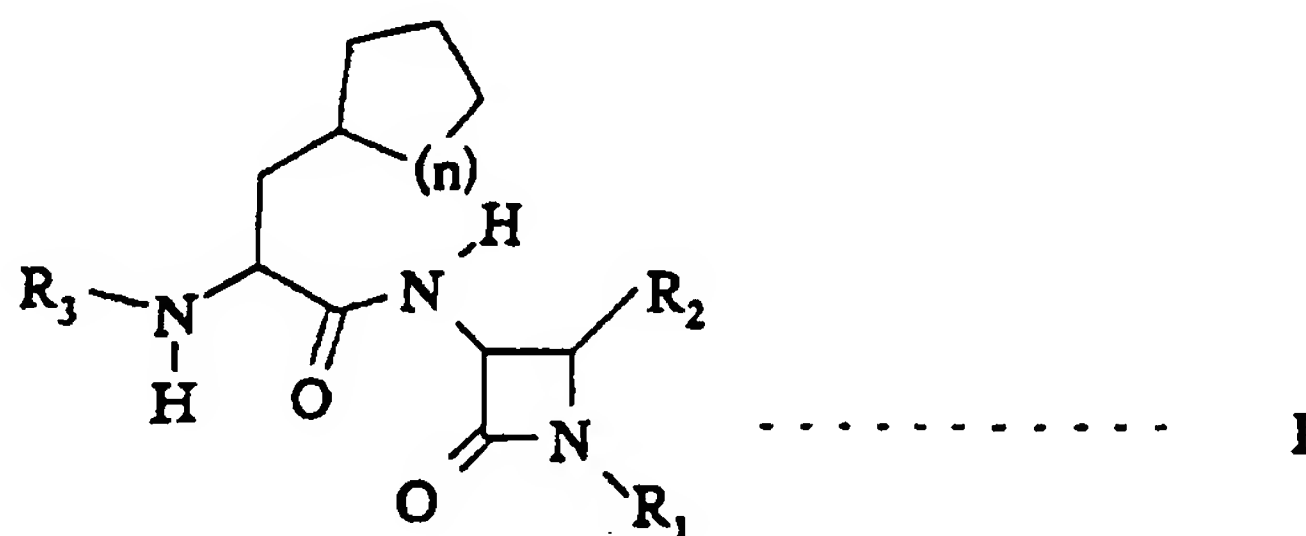


The molecular modelling of 3-substituted phenyl alanyl azetidinone suggested that the replacement of phenyl alanine with cyclohexyl alanine might increase the hydrophobic binding with cysteine proteinases. Unfortunately, there is no increase in activity as expected but it showed improved stability in plasma and good *in vivo* activity. This finding of novel 4-substituted-3-(2-amino-2-cycloalkyl methyl)- acetamido azetidin-2-one derivatives is reported in the present invention as cysteine proteinase inhibitors.

In accordance with the present invention, there are provided 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives which exhibit cysteine proteinase regulatory (e.g., inhibitory) activity with improved stability in biological fluids and which can be used for treatment of different diseases such as muscular dystrophy, myocardial infarction, bone resorption, arthritis, cancer metastasis, pulmonary emphysema, septic shock, cerebral ischemia, memory function, Alzheimer and cataract, malaria, glomerular basement membrane degradation, bacterial infection, inflammatory diseases, parasitic infections, and viral infections.

In accordance with the present invention, there are provided compounds of formula I and pharmaceutically acceptable salts thereof:

20



wherein

$n$  is 1, 2 or 3;

$R_1$  is

hydrogen; or

25

$-\text{SO}_3^-\text{M}^+$  wherein  $M$  is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, and calcium, or  $\text{N}^+(\text{R}_4)_4$

wherein  $R_4$  is a  $C_1$ - $C_6$  alkyl group;

$R_2$  is

(a) a group  $-OCOR_5$  wherein  $R_5$  is

(i) a  $C_1$ - $C_6$  alkyl group,

5 (ii) a  $C_2$ - $C_6$  alkenyl group,

(iii) a  $C_2$ - $C_6$  alkynyl group,

(iv) a  $C_3$ - $C_6$  cycloalkyl group,

(v) a phenyl group,

(vi) a naphthyl group, or

10 (vii) a monocyclic or bicyclic heterocyclic group,

which group (i), (ii), (iii), (iv), (v), (vi), or (vii) is  
unsubstituted or substituted by 1, 2 or 3  
substituents independently selected from

hydroxy,

15 halogen,

carboxy,

$C_1$ - $C_4$  alkyl (which is unsubstituted or  
substituted at least once with carboxy and/or amino),

$C_1$ - $C_2$  alkoxy,

20 amino,

cyano, and

phenyl and monocyclic or bicyclic  
heterocyclic groups, which phenyl and heterocyclic groups are unsubstituted  
or substituted by 1 or 2 substituents independently selected from

25 hydroxy,

halogen,

carboxy,

$C_1$ - $C_4$  alkyl,

$C_1$ - $C_2$  alkoxy,

30 amino, and

cyano;

or (b) a group  $-XR_5$  wherein X is selected from the group



consisting of O, S, SO, and SO<sub>2</sub>, and R<sub>5</sub> is as defined above;

R<sub>3</sub> is hydrogen, -COOR<sub>5</sub>, -COR<sub>5</sub>, -SO<sub>2</sub>R<sub>5</sub>, or -COR<sub>14</sub> wherein R<sub>5</sub> is as defined above and R<sub>14</sub> is amino group which is unsubstituted or substituted at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle, and phenyl (wherein the heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino).

In accordance with a preferred aspect of the present invention, there are provided compounds of formula I and pharmaceutically acceptable salts thereof:

wherein:

n is 1, 2 or 3

R<sub>1</sub> is

hydrogen;

-SO<sub>3</sub><sup>-</sup>M<sup>+</sup> wherein M is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, or calcium, or N<sup>+</sup>(R<sub>4</sub>)<sub>4</sub> wherein R<sub>4</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

R<sub>2</sub> is

-OCOR<sub>5</sub> wherein R<sub>5</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, and amino; or

(ii) a phenyl group which is unsubstituted or substituted at least once by 1-3 substituents selected from hydroxy, halogen, C<sub>1</sub>-C<sub>4</sub> alkyl group, C<sub>1</sub>-C<sub>2</sub> alkoxy group, and cyano;

-XR<sub>6</sub> wherein X is O, S, SO, or SO<sub>2</sub>; R<sub>6</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, amino or phenyl; or

(ii) a phenyl group which is unsubstituted or substituted at least once by 1-3 substituents selected from hydroxy, halogen, carboxy,



and C<sub>1</sub>-C<sub>4</sub> alkyl group which is unsubstituted or substituted at least once with carboxy, amino or both, C<sub>1</sub>-C<sub>2</sub> alkoxy group, cyano or heterocycle group;

R<sub>3</sub> is

hydrogen;

5           -COOR<sub>7</sub> wherein R<sub>7</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with phenyl and/or heterocycle group;

-COR<sub>8</sub> wherein R<sub>8</sub> is

10           (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle, and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino; or

15           (ii) an amino group which is unsubstituted or substituted at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino; or

20           -SO<sub>2</sub>R<sub>9</sub> wherein R<sub>9</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with heterocycle and/or phenyl; or

(ii) a C<sub>2</sub>-C<sub>4</sub> alkenyl group which is unsubstituted or substituted at least once with heterocycle and/or phenyl.

25           The pharmaceutically acceptable salts of formula I are selected from salts of sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid or p-toluenesulfonic acid.

30           Examples of C<sub>1</sub>-C<sub>6</sub> alkyl group as substituents in R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>8</sub>, or R<sub>9</sub> are straight or branched chain alkyl group having 1-6 carbon atoms such as methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, hexyl and the like.

Examples of halogen atoms as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are

fluorine, chlorine, bromine or iodine.

Examples of C<sub>2</sub>-C<sub>4</sub> alkenyl group as defined in R<sub>9</sub> are alkenyl group having 2-4 carbon atoms such as ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 3-butenyl and the like.

5            Suitable heterocyclic groups in accordance with the present invention include 5- or 6-membered aromatic or non-aromatic heterocyclic groups containing 1, 2, 3 or 4 heteroatoms selected from O, S or N, and bicyclic heterocyclic groups including a monocyclic heterocyclic as defined above which is fused to a second 5- or 6-membered carbocyclic or 5- or 6-membered  
10       heterocyclic ring.

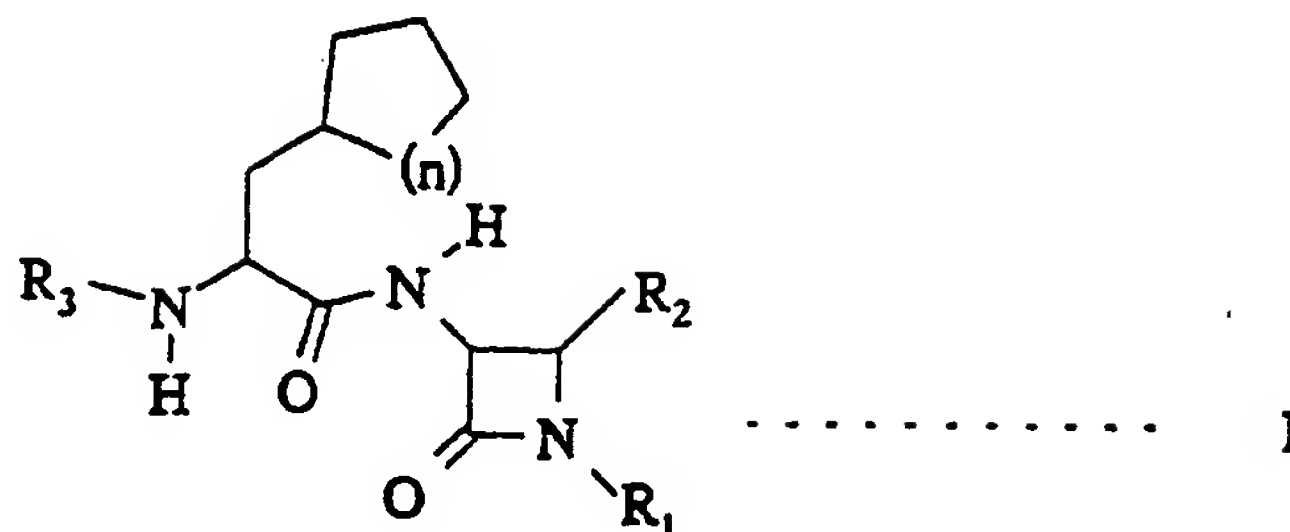
             Examples of heterocyclic group as defined in R<sub>5</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> or R<sub>9</sub> are C<sub>2</sub>-C<sub>9</sub> mono or bicyclic heterocyclic group which may have 1-3 heteroatoms selected from nitrogen, sulphur or oxygen such as thiophene, pyridine, 1,2,3-triazole, 1,2,4-triazole, quinoline, benzofuran, benzothiophene,  
15       morpholine, thiomorpholine, piperizine, piperidine and the like.

             Examples of C<sub>1</sub>-C<sub>4</sub> alkyl group as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are methyl, ethyl, propyl, 2-methyl propyl, butyl, 1,1-dimethyl ethyl and the like.

             Examples of C<sub>1</sub>-C<sub>2</sub> alkoxy group as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are methoxy or ethoxy.

20            The azetidinone nucleus carries two asymmetric carbon atoms at position 3 and 4, and can exist as 4- diastereoisomers. In general, the preferred isomer is that in which the hydrogen atoms at C3 and C4 are cis to each other for superior inhibitory activity against different cysteine proteinase such as papain, Cathepsin B, Cathepsin H, Cathepsin K and Cathepsin L.  
25       Such diastereoisomers and their racemic mixtures are also included within use of the azetidinone derivatives as cystein proteinase inhibitor.

             In accordance with preferred embodiments of the invention, there are provided 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula I:



wherein:

n is 1, 2 or 3

R<sub>1</sub> is selected from hydrogen, or sulphonic acid;

5 R<sub>2</sub> is selected from acetoxy, butyloxy, 2-carboxy ethyloxy, 2-aminoethyloxy, 2-fluoro ethoxy, phenoxy, methyl phenoxy, morpholino phenyloxy, 2-hydroxy ethylthio, phenylthio, phenylsulphonyl, 4-(2-carboxy-2-amino ethyl)-phenoxy, 4-carboxy phenoxy, 3-carboxy phenoxy, 2-pyridylthio, 4-pyridylthio, benzyloxy and the like; and

10 R<sub>3</sub> is selected from alkanoyl, aryloxy carbonyl, 3-aryl propanoyl, 3-heteroaryl propanoyl, arylmethylaminocarbonyl, 2-aryl-eth-1-en-sulphonyl, and the like.

Preferred embodiments of the present invention include the following compounds:

15 (3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexyl-methyl-acetamido)-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one;

20 (3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl)-phenoxy}-azetidin-2-one;

(3S,4R)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl)-phenoxy}-azetidin-2-one;

25 (3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-phenylthio-azetidin-2-one;

(3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo-

hexylmethyl-acetamido}-4-phenylsulfonyl-azetidin-2-one;

(3S,4S)-3-{2S-2-(benzylaminocarbonyl)amino-2-cyclo-  
hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{2S-2-(phenylethenylsulfonyl)amino-2-cyclohexylmethyl-  
5 acetamido}-4-acetoxy-azetidin-2-one;

(3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl -  
acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one;

(3S,4R)-3-(2S-2-benzyloxycarbonyl amino-2-cyclohexylmethyl-  
acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one;

10 (3S,4S)-3-{2S-2-[3-(pyridin-4-yl) propenoyl]amino-2-cyclohexylmethyl-  
acetamido}-4-phenoxy-azetidin-2-one; and

(3S,4S)-3-{2S-2-[3-(pyridin-3-yl) propenoyl]amino-2-cyclohexylmethyl-  
acetamido}-4-phenoxy-azetidin-2-one.

Compounds of formula I may be utilized for different diseases such as  
15 muscular dystrophy, myocardial infarction, bone resorption, arthritis, cancer  
metastasis, pulmonary emphysema, septic shock, cerebral ischemia, memory  
function, Alzheimer and cataract, malaria, glomerular basement membrane  
degradation, bacterial infection, inflammatory diseases, parasitic infections,  
and viral infections by regulating the cysteine proteinases in medicaments  
20 formulated with pharmaceutically acceptable carriers.

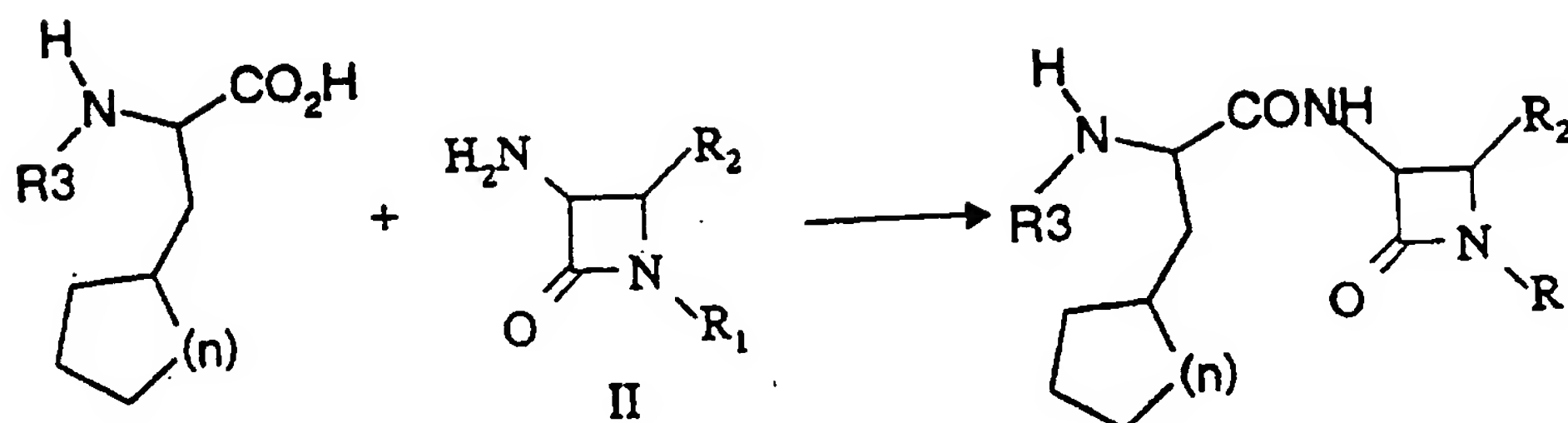
#### Brief Description of the Drawing Figure

The Figure is a graph of *in vitro* stability of compound 3 (see Example  
3) and a reference compound in rat plasma.

#### Description of Preferred Embodiments

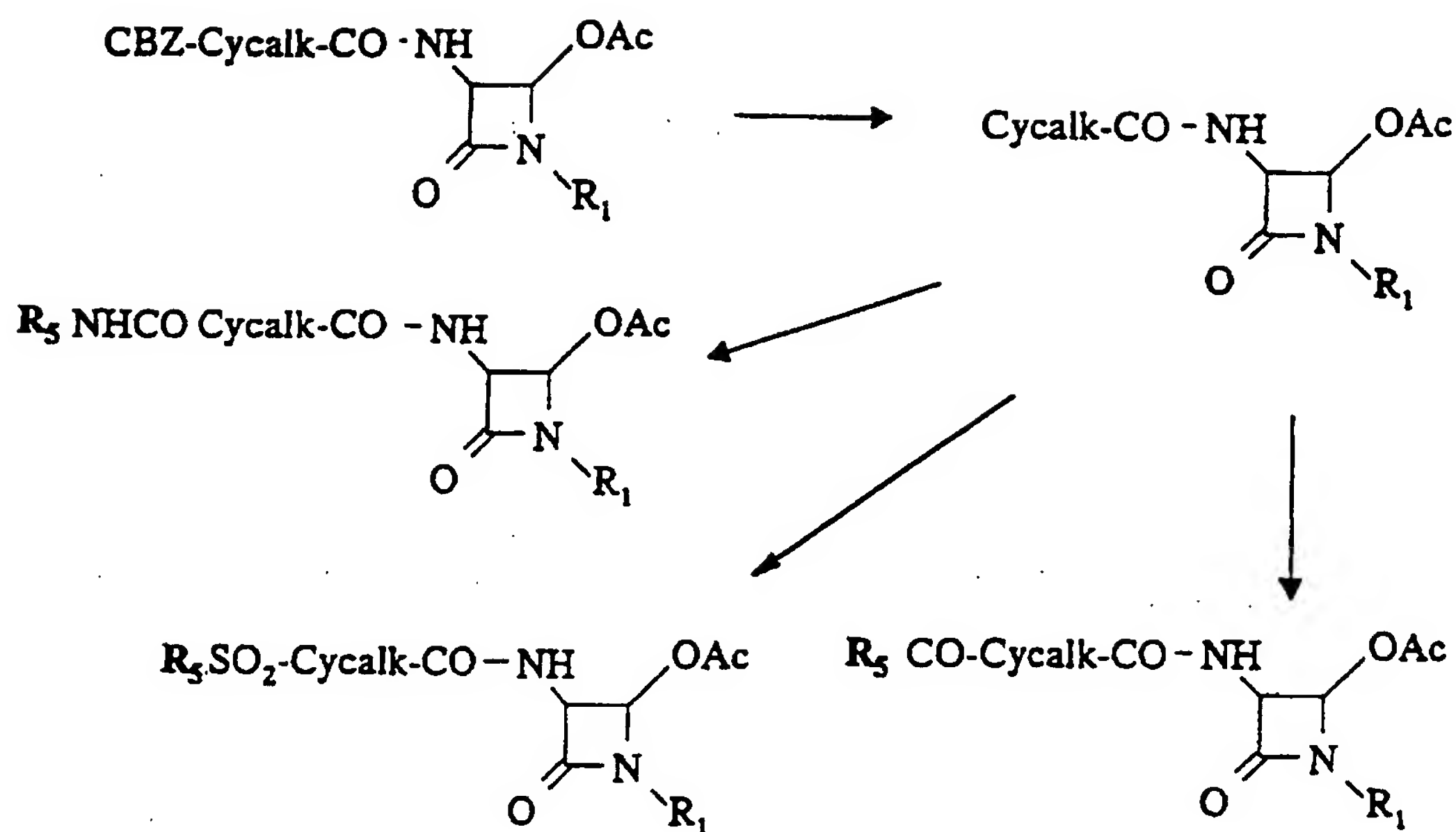
25 The present invention relates to certain  
3,4-disubstituted-azetidin-2-one derivatives having cysteine proteinase  
inhibitory activity and stability in biological fluids. The compounds of this  
invention include compounds having hydrogen, ester (OCOR<sub>5</sub>), ether (OR<sub>5</sub>),  
thioether (SR<sub>5</sub>), sulfone (SO<sub>2</sub>R<sub>5</sub>) and sulfoxide (SOR<sub>5</sub>) at position 4 and  
30 cycloalkyl alanine group at position 3 of 3-amino-azetidin-2-one (II). Certain  
derivatives of formula I are prepared by the common intermediates II by  
reacting with cycloalkyl alanine either in presence of dicyclohexylcarbodiimide

(DCC) or acid chloride in presence of base, or activated ester according to techniques known in the art.



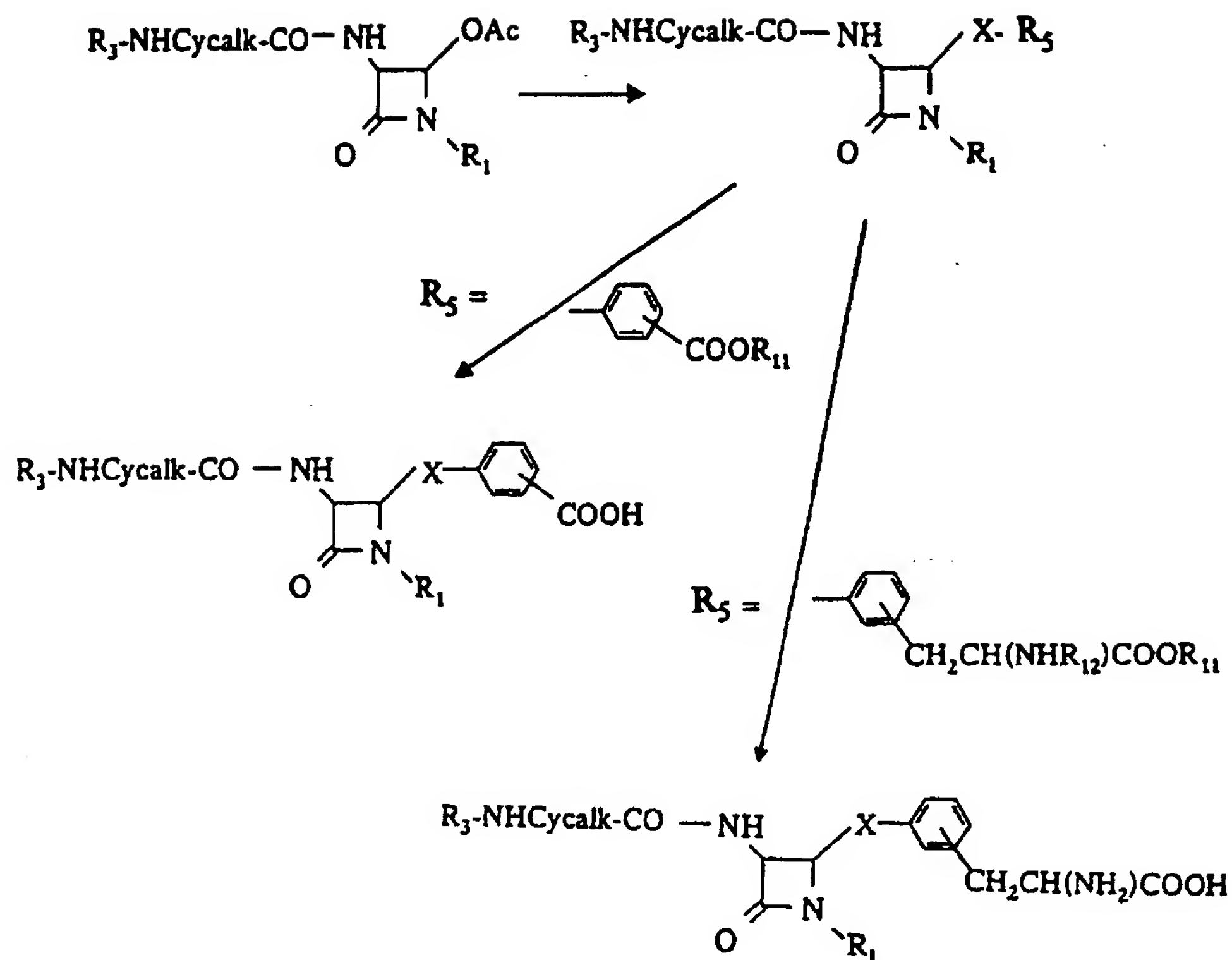
5 The preparation of compounds II is carried out by following the synthetic route as described in Eur. J. Med. Chem 1992, 27, 131-140, and Tetrahedron 1983, 39, 2577-2589, wherein R<sub>2</sub> is OCOR<sub>5</sub>, and R<sub>3</sub> is a substituent group COOR<sub>7</sub>. The definitions of R<sub>1</sub>, R<sub>5</sub> and R<sub>7</sub> are the same as defined above.

10 Certain 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula I wherein substitutions at the amino acid group are other than COOR<sub>5</sub>, such as COR<sub>5</sub> or SO<sub>2</sub>R<sub>5</sub> are prepared by following the synthetic route as shown in the scheme depicted below. The R<sub>5</sub> groups are the same as defined above.

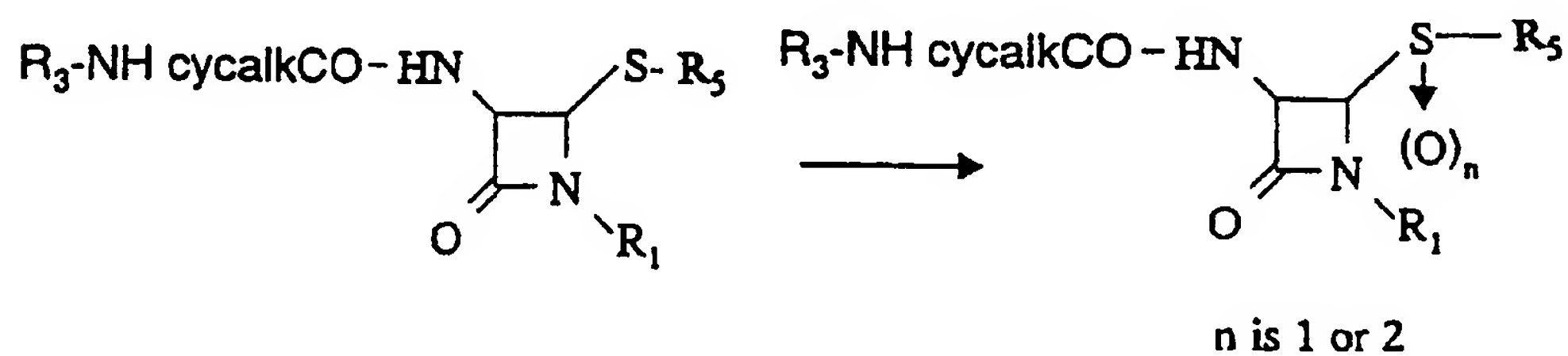


The benzyloxycarbonyl cyclohexyl alanine are desubstituted and resubstituted through amide bond by reacting with  $\text{R}_5\text{-COOH}$  either in presence of DCC or acid chloride in presence of base or anhydride in presence of base or activated ester, or through sulphonamide bond by reacting with  $\text{R}_5\text{SO}_2\text{Cl}$  in presence of base or through urea bond by reacting with  $\text{R}_5\text{NCO}$ .  $\text{R}_{11}$  is a  $\text{C}_1\text{-C}_6$  alkyl group which is unsubstituted or substituted with phenyl or heterocyclic group.

Certain 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula I wherein  $\text{R}_2$  is  $\text{XR}_5$ , wherein X is O or S, and  $\text{R}_5$  is the same as defined above, are prepared by following the synthetic route as shown below starting from compound of formula I wherein  $\text{R}_2$  is  $\text{OCOCH}_3$  by reacting with  $\text{R}_5\text{XH}$  in presence of Lewis acids such as zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, aluminum trichloride and the like or in presence of base such as sodium hydroxide. There are cases where carboxy group as substituent in  $\text{R}_5$  is substituted with  $\text{R}_{11}$  such as diphenyl methyl or 1,1-dimethyl ethyl, or amino group as substituent in  $\text{R}_5$  is substituted with  $\text{R}_{12}$  such as benzyloxy carbonyl or 1,1-dimethyl ethoxy carbonyl, or both groups as substituents in  $\text{R}_5$  together are desubstituted by hydrogenation or hydrolysis with acids.

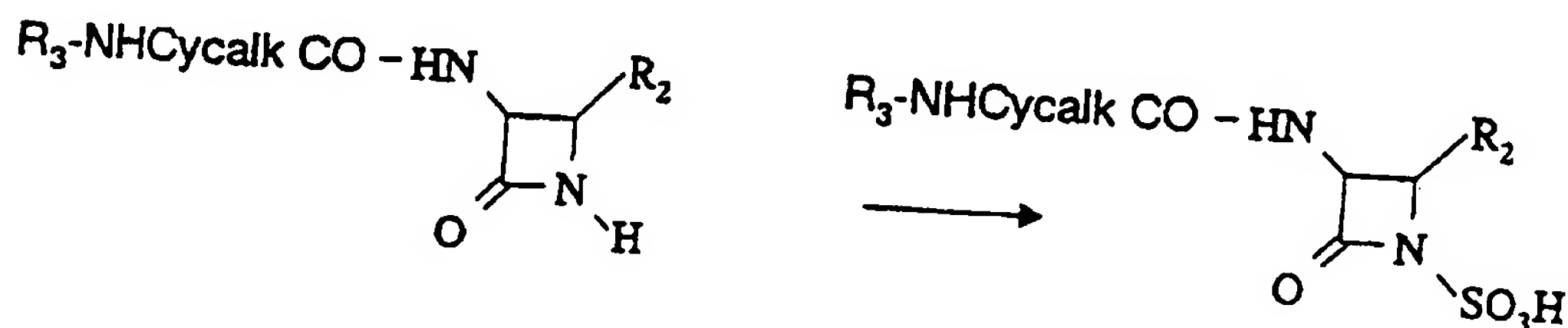


Certain 4-substituted-3-(amino-2-cyclo-alkyl methyl)-acetamido azetidin-2-one derivatives of formula I wherein  $R_2$  is  $SR_6$  are converted to  $SOR_6$  or  $SO_2R_6$  by oxidation with oxidizing agent selected from m-chloroperbenzoic acid, hydrogen peroxide, peracetic acid, potassium permanganate, magnesium dioxide and the like. The synthetic route is outlined below.





4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one derivatives of formula I wherein  $R_1$  is hydrogen can be converted to N-sulphonic acid by the sulphonation with pyridine- $\text{SO}_3$  or dimethylformamide- $\text{SO}_3$  complex by following the synthetic route as outlined below.



In the above descriptions, the reactants are reacted together with solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Wherever a base is used in a reaction, they are selected from triethylamine, pyridine, 4-dimethylaminopyridine, diisopropylethylamine, 1,5-diazabicyclo[4,3,0]non-5-ene, 1,8-diazabicyclo[5,4,0]undec-7-ene, sodium carbonate, potassium carbonate or cesium carbonate.

The solvent of choice for the reaction is selected from non-reactive solvents depending on the reactants such as benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, or the like. Solvent mixtures may also be utilized.

Suitable reaction temperatures are generally in the range of from  $-70^{\circ}\text{C}$  to  $150^{\circ}\text{C}$ . The preferred molar ratio of reactants is 1:1 to 1:5. The reaction time is in the range of from 0.5 to 72 hours, depending on the reactants.

The desubstitution of N-substituent group is carried out either by hydrogenation or by hydrolysis with appropriate acids such as hydrochloric acid, trifluoroacetic acid or acetic acid in solvent such as methanol, ethanol, propanol or ethyl acetate. The hydrogenation reaction is usually carried out  
5 in the presence of a metal catalyst, such as Pd, Pt, or Rh, under normal pressure to high pressure.

The compounds of this invention, when used alone or in combination with other drugs as an agent for treating muscular dystrophy, osteoporosis or cancer metastasis in mammals including humans, may take pharmaceutical  
10 dosage forms including parenteral preparations such as injections, suppositories, aerosols and the like, and oral preparations such as tablets, coated tablets, powders, granules, capsules, liquids and the like. Injections are generally preferred. The above preparations are formulated in a manner known in the art.

15 For the formulation of solid preparations for oral administration, an excipient, and if desired, a binder, disintegrator, lubricant, coloring agent, corrigent, flavor etc. are added to the compound of the invention, and then tablets, coated tablets, granules, powders, capsules or the like are prepared in a conventional manner.

20 For the formulation of injections, a pH adjusting agent, buffer, stabilizer, isotonic agent, local anesthetic or the like is added to the active ingredient of the invention, and injections for subcutaneous, intramuscular or intravenous administration can be prepared in the conventional manner.

For the formulation of suppositories, a base, and if desired, a surfactant  
25 are added to the active ingredient of the invention, and the suppositories are prepared in a conventional manner.

The excipients useful for solid preparations for oral administration are those generally used in the art, and the useful examples are excipients such as lactose, sucrose, sodium chloride, starches, calcium carbonate, kaolin,  
30 crystalline cellulose, methyl cellulose, glycerin, sodium alginate, gum arabic and the like, binders such as polyvinyl alcohol, polyvinyl ether, polyvinyl pyrrolidone, ethyl cellulose, gum arabic, schellac, sucrose, water, ethanol,

propanol, carboxymethyl cellulose, potassium phosphate and the like, lubricants such as magnesium stearate, talc and the like, and further include additives such as usual known colouring agents, disintegrators and the like. Examples of bases useful for the formulation of suppositories are oleaginous  
5 bases such as cacao butter, polyethylene glycol, lanolin, fatty acid triglycerides, witepsol (trademark, Dynamite Nobel Co. Ltd.) and the like. Liquid preparations may be in the form of aqueous or oleaginous suspension, solution, syrup, elixir and the like, which can be prepared by a conventional way using additives.

10 The amount of the compound I of the invention to be incorporated into the pharmaceutical composition of the invention varies with the dosage form, solubility and chemical properties of the compound, administration route, administration scheme and the like. Preferably, the amount is about 1 to 25 % (w/w) in the case of oral preparations, and about 0.1 to about 5 % (w/w) in  
15 the case of injections which are parenteral preparations.

The dosage of the compound I of the invention is suitably determined depending on the individual cases taking symptoms, age and sex of the subject and the like into consideration. Usually the dosage in the case of oral  
20 administration is about 50 to 1500 mg per day for an adult in 2 to 4 divided doses, and the dosage in the case of injection, for example, by intravenous administration is 2 ml (about 1 to 100 mg) which is administered once a day for adults wherein the injection may be diluted with physiological saline or glucose injection liquid if so desired, and slowly administered over at least 5  
25 minutes. The dosage in case of suppositories is about 1 to 1000 mg which is administered once or twice a day at an interval of 6 to 12 hours wherein the suppositories are administered by insertion into the rectum.

#### Example 1

(3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexyl - methyl-acetamido)-4-acetoxy-azetidin-2-one (1)

30 (3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (912 mg, 3.28 mmol) is hydrogenated with 1g of 10 % palladium on activated carbon in 35 ml of ethyl acetate at 50 psi hydrogen pressure at room

temperature for 1.5 hours. After removal of catalyst by filtration, desubstitution (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate is obtained.

To a solution of 2S-2-benzyloxycarbonylamino-2-cyclo-  
5 hexylmethyl-acetic acid (1.0 g, 3.28 mmol) and 1-hydroxy-benzotriazole (443 mg, 3.28 mmol) in THF (30 ml), DCC (676 mg, 3.28 mmol)/THF (10 ml) is added at 0 °C. The reaction mixture is stirred at room temperature for 2 hours and then cooled with an ice bath. The resulting DCU is removed by filtration. Then, a precooled solution of (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in  
10 ethyl acetate is added at -15 °C and the resulting mixture is stirred at a bath temperature of -15 to 5 °C for 1 hour and then at room temperature for 3 hours. After removal of solvent, the residue is dissolved in ethyl acetate, washed with cold saturated NaHCO<sub>3</sub> solution, water, brine and dried over sodium sulphate. After removal of solvent, the residue is purified by silica gel  
15 column chromatography using hexane-ethyl acetate (1:1) as eluent and the title compound is obtained.

Yield: 92 %, m.p.: 134-135 °C, FAB-MS: 432 (MH<sup>+</sup>), calcd for C<sub>22</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub> 431

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.75-1.8 (13 H, m), 2.08 (3H, s), 4.00-4.15  
20 (1H, m), 4.64 (1H, d, J=8 Hz), 5.04 (2H, m), 5.75 (1H, s), 7.30-7.45 (5H, m), 7.48 (1H, d, J=8 Hz), 8.67 (1H, d, J=8.3 Hz), 9.16 (1H, s).  
IR (KBr, cm<sup>-1</sup>): 3325, 2925, 1797, 1747, 1693, 1661, 1536, 1446, 1371, 1270, 1227.

### Example 2

25 (3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (2)

By a similar method as described in example 1, the title compound is obtained by reacting  
2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetic acid with  
30 (3S,4S)-3-amino-4-acetoxy-azetidin-2-one.

Yield: 85 %, m.p.: 166-168 °C (dec.), FAB-MS: 430 (MH<sup>+</sup>), calcd for C<sub>23</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub> 429

$^1\text{H}$  NMR ( $\text{CDCl}_3\text{-d}_6$ ),  $\delta$  (ppm): 0.80-1.80 (13H, m), 2.10 (3H, s), 2.53 (2H, t,  $J=7.5$  Hz), 2.94 (2H, t,  $J=7.5$  Hz), 4.54 (1H, m), 4.62 (1H, d,  $J=7.5$  Hz), 5.80 (1H, s), 6.18 (1H, d,  $J=8.1$  Hz), 7.10-7.35 (6H, m), 7.54 (1H, d,  $J=7.5$  Hz).

5 IR (KBr,  $\text{cm}^{-1}$ ): 3275, 2925, 1794, 1739, 1656, 1634, 1531, 1440, 1358, 1219.

### Example 3

(3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl)-phenoxy}-azetidin-2-one (3)

10 A mixture of (3S,4S)-3-{2S-2-(3-phenylpropionoyl)-amino-2-cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (550 mg, 1.28 mmol), 4-(2S-2-N-benzyloxycarbonyl-amino-2-diphenylmethoxycarbonyl-ethyl)-phenol (481 mg, 1 mmol), and zinc acetate dihydrate (300 mg, 1.36 mmol) in a mixture of benzene (18 ml) and  
15 toluene (18 ml) is refluxed for 5 hours using Dean-Stark water separator. The reaction mixture is purified by silica gel column chromatography using hexane-ethyl acetate as eluent and 200 mg of (3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-{(2S-2-N-benzyloxy-carbonylamino-2-diphenylmethoxycarbonyl-ethyl)-  
20 phenoxy}-azetidin-2-one is obtained.

$^1\text{H}$  NMR ( $\text{CDCl}_3\text{-d}_6$ ),  $\delta$  (ppm): 0.80-1.80 (13H, m), 2.45 (2H, t,  $J=7.5$  Hz), 2.87 (2H, t,  $J=7.5$  Hz), 3.01 (2H, m), 4.45-4.70 (3H, m), 5.03 (2H, s), 5.60 (1H, s), 6.50-6.90 (6H, m), 7.1-7.4 (21H, m), 7.58 (1H, d,  $J=7.5$  Hz).

25 200 mg of (3S,4S)-3-{2S-2-(3-phenylpropionoyl)-amino-2-cyclohexylmethyl-acetamido}-4-{(2S-2-N-benzyloxy-carbonyl amino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy}-azetidin-2-one is hydrogenated with 500 mg of 10 % palladium on activated carbon in 50 ml of ethyl acetate at 50 psi hydrogen pressure at room  
30 temperature for 2.5 hours. The solid is filtered and washed with ethyl acetate (3x10 ml). The solid is extracted with a mixture of water/acetonitrile (3:7) (3x20 ml). After removal of solvent, 31 mg of the title compound is obtained



as white solid.

Yield: 24 %, m.p.: 180 °C (dec.), FAB-MS: 551 (MH<sup>+</sup>), calcd for C<sub>30</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> 550

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.8 (13H, m), 2.35-2.55 (2H, m),  
5 2.70-2.90 (2H, m), 3.20-3.40 (2H, m), 4.29 (1H, m), 4.65 (1H, d, J=8 Hz),  
5.49 (1H, s), 6.83 (2H, m), 7.15-7.35 (7H, m), 8.10 (1H, d, J=8 Hz), 8.75  
(1H, d, J=8 Hz), 9.32 (1H, s).

IR (KBr, cm<sup>-1</sup>): 3385, 2925, 1791, 1750, 1681, 1647, 1623, 1556, 1522,  
1384, 1227.

10

#### Example 4

(3S,4R)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo-  
hexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl)-  
phenoxy}-azetidin-2-one (4)

To a solution of 4-(2S-2-N-benzyloxycarbonylamino-  
15 2-diphenylmethoxy carbonyl-ethyl)-phenol (7.46 g, 15.6 mmol) in acetone (80  
ml), H<sub>2</sub>O (20 ml) and 1 N NaOH (14 ml),  
(3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo-  
hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (5.51 g, 12.8 mmol) in  
acetone (100 ml) and H<sub>2</sub>O (50 ml) is slowly added at 5 °C. The mixture is  
20 stirred at 5 °C for 2 hours. After removal of solvent, the residue is dissolved  
in ethyl acetate, washed with water, brine and dried over sodium sulphate.  
After removal of solvent, the residue is recrystallized from methanol/ethyl  
acetate/hexane and 2.1 g of (3S,4R)-3-{2S-2-(3-phenylpropionoyl)amino-2-  
cyclohexylmethyl-acetamido}-4-{(2S-2-N-benzyloxy-  
25 carbonylamino-2-diphenylmethoxy carbonyl-ethyl)-phenoxy}-  
azetidin-2-one is obtained as white solid.

910 mg of (3S,4R)-3-{2S-2-(3-phenylpropionoyl)amino-  
2-cyclohexylmethyl-acetamido}-4-{(2S-2-N-benzyloxy-  
carbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy}-  
30 azetidin-2-one is hydrogenated with 2 g of 10 % palladium on activated  
carbon in a mixture of ethyl acetate (50 ml), THF (50 ml) and ethanol (20 ml)  
at 50 psi hydrogen pressure at room temperature for 4 hours. The solid is

filtered and washed with ethyl acetate (3x20 ml). The solid is extracted with a mixture of water/acetonitrile (4:6) (2x50 ml). After removal of solvent, the resulting solid is washed with acetonitril and 265 mg of the title compound is obtained as white solid.

5 Yield: 45 %, m.p.: 161-162 °C, FAB-MS: 551 (MH<sup>+</sup>), calcd for C<sub>30</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub> 550

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.8 (13H, m), 2.35-2.50 (2H, m), 2.7-2.9 (2H, m), 3.2-3.4 (2H, m), 4.35 (1H, m), 5.27 (1H, dd, J=8, 3 Hz), 5.65 (1H, d, J=3 Hz), 6.82 (2H, m), 7.05-7.30 (7H, m), 7.94 (1H, d, J=8 Hz), 8.64 (1H, d, J=8 Hz), 9.28 (1H, s).

10 IR (KBr, cm<sup>-1</sup>): 3400, 3290, 2925, 1771, 1643, 1555, 1506, 1396, 1230.

#### Example 5

(3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-phenylthio-azetidin-2-one (5)

15 To a solution of thiophenol (149 mg, 1.36 mmol) in THF (5 ml), water (5 ml) and 1 N NaOH (1.2 ml), (3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (387 mg, 0.9 mmol) in acetone (10 ml) and THF (5 ml) is added at 5 °C. The mixture is stirred at 5 °C for 1  
20 hour and then at room temperature for 1 hour. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulphate. After removal of solvent, the residue is purified by recrystallization from THF-ethyl acetate-hexane and 164 mg of title compound is obtained.

25 Yield: 38 %, m.p.: 198-200 °C, FAB-MS: 480 (MH<sup>+</sup>), calcd for C<sub>27</sub>H<sub>33</sub>N<sub>3</sub>O<sub>3</sub>S 479

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.7 (13H, m), 2.45 (2H, m), 2.80 (2H, m), 4.34 (0.85H, m), 4.45 (0.15H, m), 4.54 (0.85H, dd, J=8.5, 2.0 Hz), 4.92 (0.85 H, d, J=2.0 Hz), 5.25-5.35 (0.3H, m), 7.10-7.50 (10H, m), 7.98 (0.15H, d, J=8.1 Hz), 8.05 (0.85 H, d, J=8.1 Hz), 8.71 (0.85H, d, J=8.6 Hz), 8.83 (0.15H, d, J=8.6 Hz), 9.00 (1H, s).

30 IR (KBr, cm<sup>-1</sup>): 3270, 2905, 1763, 1735, 1634, 1523, 1436, 1367, 1222.



Example 6

(3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-phenylsulfonyl-azetidin-2-one (6)

A mixture of (3S,4SR)-3-{2S-2-(3-phenylpropionoyl)-  
5 amino-2-cyclohexylmethyl-acetamido}-4-phenylthio-  
azetidin-2-one (100 mg, 0.208 mmol) obtained in example 5, and  $\text{KMnO}_4$  (50  
mg, 0.32 mmol) in acetic acid (10 ml) and  $\text{H}_2\text{O}$  (2 ml) is stirred at 5 °C for 1  
hour and then room temperature for 1 hour. One drop of  $\text{H}_2\text{O}_2$  (30% aq) is  
added. The reaction mixture is partitioned between ethyl acetate and water,  
10 the organic layer is washed with water, saturated  $\text{NaHCO}_3$ , water, brine and  
dried over  $\text{Na}_2\text{SO}_4$ . After removal of the solvent, solid is washed with ether  
and 78 mg of the title compound is obtained. Yield: 73 %, m.p.: 170 °C  
(dec.), FAB-MS: 512 ( $\text{MH}^+$ ), calcd for  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_5\text{S}$  511  
 $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ),  $\delta$  (ppm): 0.6-1.7 (13H, m), 2.45 (2H, m), 2.80 (2H, m),  
15 4.30 (0.85H, m), 4.50 (0.15H, m), 4.87 (0.85H, dd,  $J=8.2$  & 2.1 Hz), 4.95  
(0.85H, d,  $J=2.1$  Hz), 5.20 (0.15H, d,  $J=4.6$  Hz), 5.51 (0.15H, m), 7.22 (5H,  
m), 7.60-8.00 (5H, m), 8.05 (1H, d,  $J=8.3$  Hz), 8.48 (0.15H, d,  $J=8.4$  Hz),  
8.71 (0.85H, d,  $J=8.4$  Hz), 9.31 (0.85H, s), 9.40 (0.15H, s).  
IR (KBr,  $\text{cm}^{-1}$ ): 3280, 2905, 1779, 1640, 1517, 1440, 1301.

20

Example 7

(3S,4S)-3-{2S-2-(benzylaminocarbonyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (7)

(3S,4S)-3-{2S-2-(benzyloxycarbonyl)amino-2-cyclo-  
hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (from example 1) (216 mg,  
25 0.5 mmol) is hydrogenated with 400 mg of 10 % palladium on activated carbon  
in ethyl acetate (15 ml) and THF (7 ml) at 50 psi hydrogen pressure at room  
temperature for 3 hours. After removal of catalyst by filtration, desubstituted  
(3S,4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-  
2-one in ethyl acetate/THF is cooled to -15 °C and then benzyl isocyanate  
30 (106 mg, 0.8 mmol) is added. The reaction mixture is stirred at -10 to 0 °C for  
1 hour and room temperature for 1 hour. After removal of solvent, the residue  
is dissolved in ethyl acetate, washed with cold saturated  $\text{NaHCO}_3$  solution,

water, brine and dried over sodium sulphate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate (1:2) as eluent and the title compound is obtained.

Yield: 74 %, m.p.: 192-194 °C, FAB-MS: 431 (MH<sup>+</sup>), calcd for C<sub>22</sub>H<sub>30</sub>N<sub>4</sub>O<sub>5</sub>  
5 430

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.8 (13H, m), 2.07 (3H, s), 4.15-4.30 (3H, m), 4.64 (1H, d, J=8.5Hz), 5.74 (1H, s), 6.15 (1H, d, J=8.6 Hz), 6.46 (1H, m), 4.20-4.35 (5H, m), 8.71 (1H, d, J=8.5 Hz), 9.16 (1H, s).

IR (KBr, cm<sup>-1</sup>): 3325, 2905, 1789, 1732, 1653, 1628, 1554, 1526, 1440, 1357,  
10 1223.

#### Example 8

(3S,4S)-3-{2S-2-(phenylethenylsulfonyl)amino-2-cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (8)

(3S,4S)-3-{2S-2-(benzyloxycarbonyl)amino-2-cyclo-  
15 hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one (from example 1) (216 mg, 0.5 mmol) is hydrogenated with 400 mg of 10 % palladium on activated carbon in ethyl acetate (15 ml) and THF (7 ml) at 50 psi hydrogen pressure at room temperature for 3 hours. After removal of catalyst by filtration, desubstituted (3S,4S)-3-(2S-2-amino-2-

20 cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one in ethyl acetate/THF is cooled to -15 °C and then triethylamine (50 mg, 0.5 mmol) and benzyl isocyanate (106 mg, 0.8 mmol) is added. The reaction mixture is stirred at -10 to 0 °C for 1 hour and at 5 °C overnight. After removal of solvent, the residue is dissolved in ethyl acetate, washed with cold saturated NaHCO<sub>3</sub> solution,  
25 water, brine and dried over sodium sulphate. After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as eluent and the title compound is obtained.

Yield: 35 %, m.p.: 77 °C (dec.), FAB-MS: 464 (MH<sup>+</sup>), calcd for C<sub>22</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>S  
30 463

<sup>1</sup>H NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.8 (13H, m), 2.02 (3H, s), 3.70-3.85 (1H, m), 4.61 (1H, d, J=7.6 Hz), 5.54 (1H, s), 6.99 (1H, d, J=15.5 Hz), 7.32 (1H, d, J=15.5 Hz), 7.40-7.50 (3H, m), 7.60-7.70 (2H, m), 7.82 (1H, d, J=7.6 Hz),

8.80 (1H, d, J=8.0 Hz), 9.18 (1H, s).

IR (KBr,  $\text{cm}^{-1}$ ): 3295, 2905, 1778, 1744, 1659, 1521, 1441, 1317, 1222.

#### Example 9

5 (3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-  
acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one (9A) and (3S,4R)-3-(2S-2-  
benzyloxycarbonyl amino-2-cyclohexylmethyl-acetamido)-4-(3-methyl-  
phenoxy)-azetidin-2-one (9B)

To a solution of 3-methyl-phenol (81 mg, 0.75 mmole) in acetone (2ml) and 1N NaOH (0.6 ml, 0.6 mmole), (3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-4-acetoxy-azetidin-2-one (216mg, 0.5 mmole) in THF (4 ml) and  $\text{H}_2\text{O}$  (1ml) is added at 0°C. The mixture is stirred at 0°C for 1 hour and then at room temperature for 30 min. After removal of solvent, the residue is dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate.  
15 After removal of solvent, the residue is purified by silica gel column chromatography using hexane-ethyl acetate as eluent. 110 mg of (3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexyl-methyl-acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one (9A) and 40 mg of (3S,4R)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl-acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one (9B) are obtained.  
20

For (9A):

Yield: 46%

m.p.: 184-185.5°C

25  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) ,  $\delta$  (ppm): 0.7-1.8 (13H, m), 2.26 (3H,s), 4.0-4.2 (1H, m), 4.64 (1H, d, J=8.5 Hz), 5.05 (2H, m), 5.50 (1H,s), 6.6-6.7 (2H, m), 6.83 (1H,d, J=7.3 Hz), 7.1-7.4 (6H,m), 7.52 (1H,d, J=8 Hz), 8.82 (1H,d, J=8.5 Hz), 9.28 (1H, s).

For (9B):

Yield: 17%

30 m.p.: 178-179°C

$^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ) $\delta$  (ppm): 0.7-1.8 (13H, m), 2.24 (3H,s), 4.0-4.2 (1H, m), 5.01 (2H, m), 5.33 (1H,m), 5.68 (1H,d, J=3.7 Hz), 6.6-6.85 (3H,m), 7.1-7.4

(7H, m), 8.61 (1H, d, J=9.2 Hz), 9.23 (1H, s).

#### Example 10

(3S,4S)-3-{2S-2-[3-(pyridin-4-yl) propenoyl]amino-2-cyclohexylmethyl-acetamido}-4-phenoxy-azetidin-2-one (10)

5 The title compound was synthesized by the reaction of succinimidyl 3-(pyridin-4-yl) propanoic acid with (3S,4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one in DMF.

Yield: 43%

m.p.: 145-147°C

10 <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.7-1.8 (13H, m), 4.35-3.50 (1H, m), 4.66 (1H, d, J=8.3 Hz), 5.55 (1H, s), 6.86-7.55 (9H, m), 8.54 (1H, d, J=8.0 Hz), 8.60 and 8.65 (2H, 2s), 8.93 (1H, d, J=8.4 Hz), 9.31 (1H, s).

#### Example 11

15 (3S,4S)-3-{2S-2-[3-(pyridin-3-yl) propenoyl]amino-2-cyclohexylmethyl-acetamido}-4-phenoxy-azetidin-2-one (11)

The title compound was synthesized by the reaction of succinimidyl 3-(pyridin-3-yl) propenoic acid with (3S,4S)-3-(2S-2-amino-2-cyclohexylmethyl-acetamido)-4-phenoxy-azetidin-2-one in DMF.

Yield: 47%

20 m.p.: 148-150°C

<sup>1</sup>H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 0.3-1.8 (13H, m), 4.42-4.54 (1H, m), 4.67 (1H, d, J=8.0 Hz), 5.55 (1H, s), 6.82-7.56 (8H, m), 7.99 (1H, d, J=7.9 Hz), 8.45 (1H, d, J=8.0 Hz), 8.56 (1H, d, J=4.7 Hz), 8.77 (1H, s), 8.92 (1H, d, J=8.5 Hz), 9.31 (1H, s).

25 Testing of inhibitors for inhibition of Cathepsin B and L

#### Test Example 1

##### In vitro assay procedure for cathepsin B

The compounds of formula I are tested for inhibition of cathepsin B using the known method (A.J. Barret et al., Biochem. J. 1982, 201, 189-198).

30 To 170 μl of an enzyme-buffer mixture (enzyme: recombinant rat cathepsin B, diluted to give approximate 10 Fluorescence units/min, buffer: 56 mM sodium acetate, 1.124 mM EDTA, 10 mM DTT, pH 5.1) 10 μL of inhibitor (dissolved

in DMSO) is added. After 10 min of incubation at room temperature, a 20  $\mu$ l of 5 mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) is added to initiate reaction. Reading is followed up for 10 min on a Fluoroskan fluorescence reader (excitation at 380 nm, emission at 460 nm).

- 5 A plot of percentage of inhibition vs inhibitor concentration is obtained, and  $IC_{50}$  is determined using a linear regression calculation (concentration of inhibitor which will give 50% inhibition).

#### Test Example 2

##### In vitro assay procedure for cathepsin L

- 10 To 170  $\mu$ l of enzyme-buffer mixture (enzyme: recombinant rat cathepsin L, diluted to give approximate 15 Fluorescence units/min, buffer: 58.8 mM sodium citrate, 1.18 mM EDTA, 235 mM sodium chloride, 5 mM DTT, pH 5.0) 10  $\mu$ L of inhibitor (dissolved in DMSO) is added. After 10 min of incubation at room temperature, 20  $\mu$ l of 1 mM substrate  
15 (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) is added to initiate reaction. Reading is followed up for 10 min on a Fluoroskan fluorescence reader (excitation at 380 nm emission at 460 nm).

- A plot of percentage of inhibition vs inhibitor concentration is obtained, and  $IC_{50}$  is determined using a linear regression calculation (concentration of  
20 inhibitor which will give 50% inhibition).

Table 1. In vitro inhibitory activity of monobactam compounds on cysteine proteases

Example No.	$IC_{50}$ ( $\mu$ M)	
	Cathepsin B	Cathepsin L
25		
1	8.71	0.78
2	11.6	2.32
3	34	1.82
30		
4	9.2	1.8
5	10.4	0.016
6	29	0.078



7	11.6	2.30
8	11	2.16
9A	6.9	0.083
9B	0.25	0.003
10	1	0.4
11	2.2	0.43

### In vitro stability test in rat plasma

10           The testing compound is added to Rat plasma/phosphate buffer  
(pH=7.4) at 37 °C (the final concentration is 200 µg/ml) and the resulting  
solution kept at 37 °C. Samples are taken at 0, 0.5, 1, 2, 4, and 6 hours. 500  
µl of sample is taken in duplicate for each time. To the 500 µl of the sample,  
500 µl of ice-cold acetonitrile is added to precipitate the protein, and the  
15           product is then vortexed for 30 seconds and centrifuged at 5000 rpm for 10  
mins. The supernatant is removed and to it is added 2.0 ml of methylene  
chloride. The mixture is vortexed for 30 seconds and then centrifuged at 5000  
rpm for 10 mins. The upper layer is directly injected onto the HPLC for  
analysis. The results are shown in the Figure.

### In vivo inhibition test for cathepsin B and L

The in vivo inhibition of cathepsin B and L are tested according to the known method (T. Towatari et al, FEBS, 1991, 280, 311-315). Inhibitor is injected intraperitoneally into rodents as a solution in saline containing DMSO or DMSO:PEG400 (1:1) at the doses indicated in Table 2. The rodents are killed after 6 hours, and the liver is perfused with ice-cold saline, and chilled on ice. Sample of 4 g of liver are homogenized in 7 volumes of 0.25 M sucrose. The homogenate is centrifuged at 800 g for 15 min. and the supernatant is centrifuged at 12,000 g for 30 min. The precipitate (crude mitochondrial-lysosomal fraction; ML fraction) is suspended in 2 ml of 0.05 M acetate buffer, pH 5.0, and then freeze-thawed for measurements of cathepsin B and L.

**Table 2.** In vivo inhibition of the inhibitors for cathepsin B and L.

Compd.	Species	Dosage (mg/kg)	Inhibition <sup>a</sup>	
			Cathepsin B	Cathepsin L
Ref. Compd. <sup>b</sup>	rat	30	37%	30%
	rat	70	56%	55%
Compd. 3	mouse	50	65%	55%

a. Values are means for 3 animals.

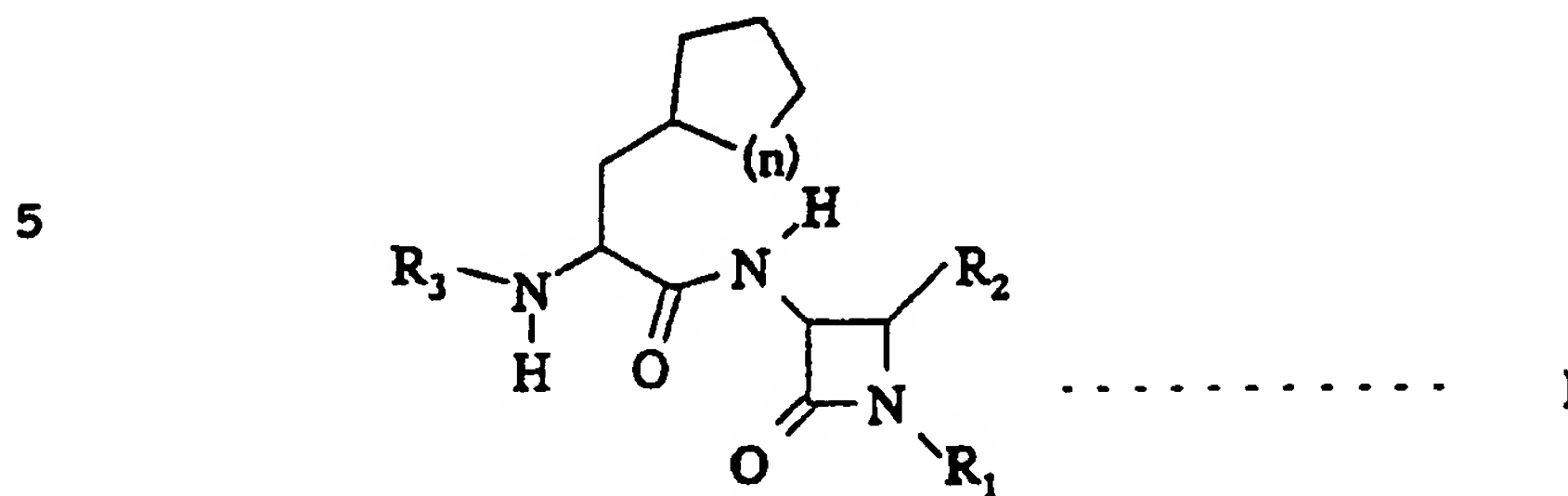
- 5 b. Reference compound is (3S,4S)-3-{N-(3-phenyl-propionoyl)-L-phenylalanyl} amino-4-(4-(2S-2-amino-2-carboxy ethyl)-phenoxy)-azetidin-2-one.

10 Although the compounds, the methods of treatment and the methods of making the compounds in accordance with the present invention have been described in connection with preferred embodiments, it will be appreciated by those skilled in the art that modifications not specifically described may be made without departing from the spirit and scope of the invention defined in the following claims.



## Claims:

1. A 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido azetidin-2-one compound of formula I, or a pharmaceutically acceptable salt thereof:



wherein

n is 1, 2 or 3;

R<sub>1</sub> is

hydrogen; or

10        -SO<sub>3</sub><sup>-</sup>M<sup>+</sup> wherein M is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, and calcium, or N<sup>+</sup>(R<sub>4</sub>)<sub>4</sub> wherein R<sub>4</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

R<sub>2</sub> is

(a) a group -OCOR<sub>5</sub> wherein R<sub>5</sub> is

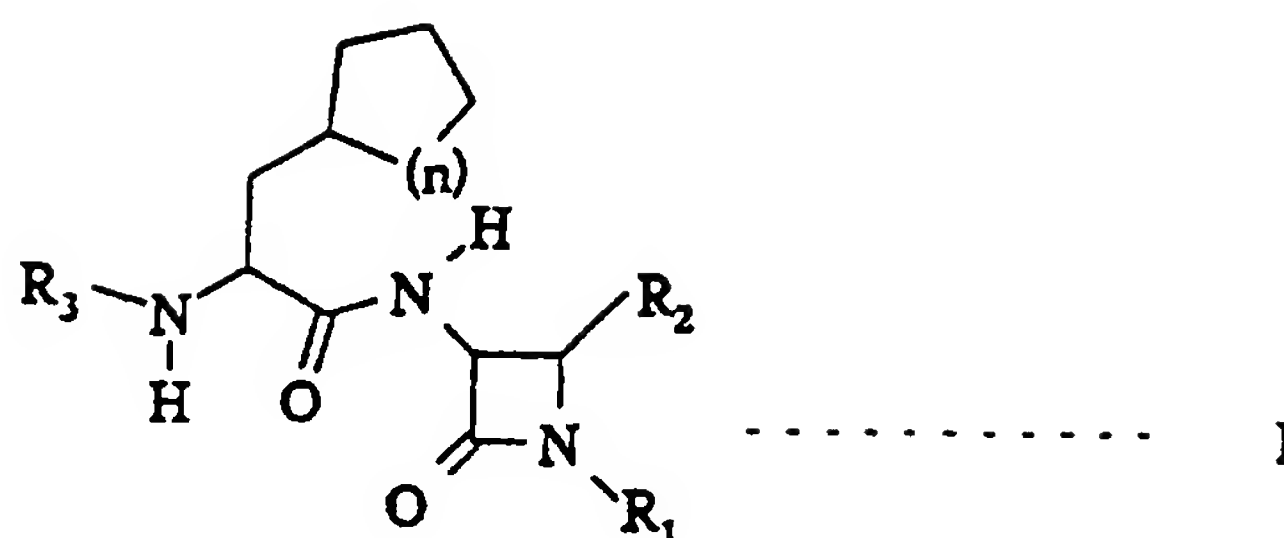
15

- (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group,
- (ii) a C<sub>2</sub>-C<sub>6</sub> alkenyl group,
- (iii) a C<sub>2</sub>-C<sub>6</sub> alkynyl group,
- (iv) a C<sub>3</sub>-C<sub>6</sub> cycloalkyl group,
- (v) a phenyl group,
- (vi) a naphthyl group, or
- (vii) a monocyclic or bicyclic heterocyclic group,

20

which group (i), (ii), (iii), (iv), (v), (vi), or (vii) is unsubstituted or substituted by 1, 2 or 3 substituents independently selected from

- hydroxy,  
halogen,  
carboxy,  
C<sub>1</sub>-C<sub>4</sub> alkyl (which is unsubstituted or  
5 substituted at least once with carboxy and/or amino),  
C<sub>1</sub>-C<sub>2</sub> alkoxy,  
amino,  
cyano, and  
phenyl and monocyclic or bicyclic  
10 heterocyclic groups, which phenyl and heterocyclic groups are unsubstituted  
or substituted by 1 or 2 substituents independently selected from  
hydroxy,  
halogen,  
carboxy,  
15 C<sub>1</sub>-C<sub>4</sub> alkyl,  
C<sub>1</sub>-C<sub>2</sub> alkoxy,  
amino, and  
cyano;  
or (b) a group -XR<sub>5</sub> wherein X is selected from the group  
20 consisting of O, S, SO, and SO<sub>2</sub>, and R<sub>5</sub> is as defined above;  
R<sub>3</sub> is hydrogen, -COOR<sub>5</sub>, -COR<sub>5</sub>, -SO<sub>2</sub>R<sub>5</sub>, or -COR<sub>14</sub> wherein R<sub>5</sub> is as  
defined above and R<sub>14</sub> is amino group which is unsubstituted or substituted  
at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at  
least once with 1 or 2 substituents selected from hydroxy, halogen, cyano,  
25 amino, heterocycle, and phenyl (wherein the heterocycle or phenyl is  
unsubstituted or substituted at least once by 1 or 2 substituents selected from  
halogen, hydroxy, cyano, carboxy and amino).
2. A 4-substituted-3-(2-amino-2-cycloalkyl methyl)-acetamido  
azetidin-2-one compound of formula I, or a pharmaceutically acceptable salt  
30 thereof:



wherein

$n$  is 1, 2 or 3

$R_1$  is

5 hydrogen;

$-\text{SO}_3^- \text{M}^+$  wherein  $\text{M}$  is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, or calcium, or  $\text{N}^+(\text{R}_4)_4$  wherein  $\text{R}_4$  is a  $\text{C}_1$ - $\text{C}_6$  alkyl group;

$R_2$  is

10  $-\text{OCOR}_5$  wherein  $\text{R}_5$  is

(i) a  $\text{C}_1$ - $\text{C}_6$  alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, and amino; or

(ii) a phenyl group which is unsubstituted or substituted  
15 at least once by 1-3 substituents selected from hydroxy, halogen,  $\text{C}_1$ - $\text{C}_4$  alkyl group,  $\text{C}_1$ - $\text{C}_2$  alkoxy group, and cyano;

$-\text{XR}_6$  wherein  $\text{X}$  is O, S, SO, or  $\text{SO}_2$ ;  $\text{R}_6$  is

(i) a  $\text{C}_1$ - $\text{C}_6$  alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy,  
20 halogen, amino or phenyl; or

(ii) a phenyl group which is unsubstituted or substituted at least once by 1-3 substituents selected from hydroxy, halogen, carboxy, and  $\text{C}_1$ - $\text{C}_4$  alkyl group which is unsubstituted or substituted at least once with carboxy, amino or both,  $\text{C}_1$ - $\text{C}_2$  alkoxy group, cyano or heterocycle group;

25  $R_3$  is

hydrogen;

-COOR<sub>7</sub> wherein R<sub>7</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with phenyl and/or heterocycle group;

-COR<sub>8</sub> wherein R<sub>8</sub> is

5 (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle, and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino; or

10 (ii) an amino group which is unsubstituted or substituted at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from  
15 halogen, hydroxy, cyano, carboxy and amino; or

-SO<sub>2</sub>R<sub>9</sub> wherein R<sub>9</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with heterocycle and/or phenyl; or

(ii) a C<sub>2</sub>-C<sub>4</sub> alkenyl group which is unsubstituted or  
20 substituted at least once with heterocycle and/or phenyl.

3. A compound or salt as recited in claim 2, wherein halogen atoms as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are fluorine, chlorine, bromine or iodine.

4. A compound or salt as recited in claim 2, wherein C<sub>2</sub>-C<sub>4</sub> alkenyl group as defined in R<sub>9</sub> are alkenyl group having 2-4 carbon atoms.

25 5. A compound or salt as recited in claim 2, wherein heterocyclic group as defined in R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> or R<sub>9</sub> are C<sub>2</sub>-C<sub>9</sub> mono or bicyclic heterocyclic group which have 1-3 heteroatoms selected from nitrogen, sulfur and oxygen.

6. A compound or salt as recited in claim 2, wherein C<sub>1</sub>-C<sub>4</sub> alkyl group as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are branched or straight alkyl chain selected  
30 from methyl, ethyl, propyl, 2-methyl propyl, butyl and 1,1-dimethyl ethyl.

7. A compound or salt as recited in claim 2, wherein C<sub>1</sub>-C<sub>2</sub> alkoxy group as substituents in R<sub>5</sub>, R<sub>6</sub>, or R<sub>9</sub> are methoxy or ethoxy.

8. A compound or salt as recited in claim 2, wherein said compound is selected from the diastereoisomers and their racemates related to the azetidinone nucleus.

5 9. A compound or salt as recited in claim 2, wherein said compound is selected from the D, L isomers or racemates of cycloalkyl alanine.

10. A salt as recited in claim 2, wherein said salt comprises a component selected from sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid.

10 11. A compound as recited in claim 2, wherein C<sub>1</sub>-C<sub>6</sub> alkyl group as substituents in R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>8</sub>, or R<sub>9</sub> are straight or branched chain alkyl groups having 1-6 carbon atoms.

15 12. A compound or salt as recited in claim 11, wherein C<sub>1</sub>-C<sub>6</sub> alkyl group as substituents in R<sub>4</sub>, R<sub>5</sub>, R<sub>6</sub>, R<sub>8</sub>, or R<sub>9</sub> are selected from methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, and hexyl.

13. A compound or salt as recited in claim 4, wherein C<sub>2</sub>-C<sub>4</sub> alkenyl group as defined in R<sub>9</sub> are selected from ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, and 3-butenyl.

20 14. A compound or salt as recited in claim 5, wherein heterocyclic group as defined in R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub> or R<sub>9</sub> are selected from thiophene, pyridine, 1,2,3-triazole, 1,2,4-triazole, quinoline, benzofuran, benzothiophene, morpholine, thiomorpholine, piperazine, and piperidine.

15. A compound or salt as recited in claim 8, wherein said compound is selected from the isomers in which C3 and C4 are cis to each other.

25 16. A pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 and a pharmaceutically acceptable carrier.

17. A method of treatment of osteoporosis in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating osteoporosis, and a pharmaceutically acceptable carrier.

30

18. A method of treatment of muscular dystrophy in a patient in need

of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating muscular dystrophy, and a pharmaceutically acceptable carrier.

5           19. A method of treatment of inflammatory disease in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating inflammatory disease, and a pharmaceutically acceptable carrier.

10           20. A method of treatment of myocardial infarction in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating myocardial infarction, and a pharmaceutically acceptable carrier.

15           21. A method of treatment of arthritis in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating arthritis, and a pharmaceutically acceptable carrier.

20           22. A method of treatment of pulmonary emphysema in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating pulmonary emphysema, and a pharmaceutically acceptable carrier.

25           23. A method of treatment of septic shock in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating septic shock, and a pharmaceutically acceptable carrier.

30           24. A method of treatment of cerebral ischemia in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in

an amount which is effective for treating cerebral ischemia, and a pharmaceutically acceptable carrier.

25. A method for improvement of memory function in a patient in need of such improvement, comprising administering to said patient a  
5 pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for improving memory function, and a pharmaceutically acceptable carrier.

26. A method of treatment of parasitic infection in a patient in need of such treatment, comprising administering to said patient a pharmaceutical  
10 composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating parasitic infection, and a pharmaceutically acceptable carrier.

27. A method of treatment of cataract in a patient in need of such treatment, comprising administering to said patient a pharmaceutical  
15 composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating cataract, and a pharmaceutically acceptable carrier.

28. A method of treatment of malaria in a patient in need of such treatment, comprising administering to said patient a pharmaceutical  
20 composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating malaria, and a pharmaceutically acceptable carrier.

29. A method of treatment of glomerular basement membrane degradation in a patient in need of such treatment, comprising administering  
25 to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating glomerular basement membrane degradation, and a pharmaceutically acceptable carrier.

30. A method of treatment of viral infection in a patient in need of such  
30 treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating viral infection, and a pharmaceutically

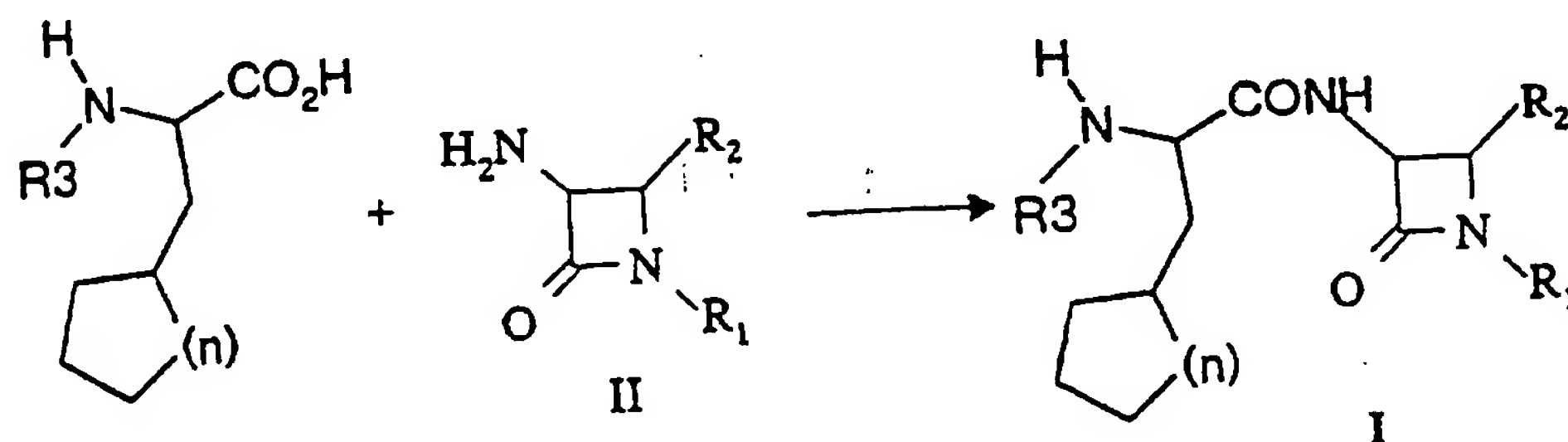


acceptable carrier.

31. A method of regulating cysteine protease in a patient in need of such regulating, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating regulating cysteine protease, and a pharmaceutically acceptable carrier.

32. A method of treatment of cancer metastasis in a patient in need of such treatment, comprising administering to said patient a pharmaceutical composition comprising a compound or salt as recited in claim 1 or claim 2 in an amount which is effective for treating cancer metastasis, and a pharmaceutically acceptable carrier.

33. A method of preparing a compound of formula I, comprising reacting a compound according to formula II with a substituted cycloalkyl alanine in the presence of at least one member selected from the group consisting of dicyclohexylcarbodiimide, acid chloride, base and activated ester:



wherein

n is 1, 2 or 3;

R<sub>1</sub> is

hydrogen; or

-SO<sub>3</sub><sup>-</sup>M<sup>+</sup> wherein M is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, and calcium, or N<sup>+</sup>(R<sub>4</sub>)<sub>4</sub> wherein R<sub>4</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

R<sub>2</sub> is

(a) a group -OCOR<sub>5</sub> wherein R<sub>5</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group,

- (ii) a C<sub>2</sub>-C<sub>6</sub> alkenyl group,  
(iii) a C<sub>2</sub>-C<sub>6</sub> alkynyl group,  
(iv) a C<sub>3</sub>-C<sub>6</sub> cycloalkyl group,  
(v) a phenyl group,  
5 (vi) a naphthyl group, or  
(vii) a monocyclic or bicyclic heterocyclic group,

which group (i), (ii), (iii), (iv), (v), (vi), or (vii) is  
unsubstituted or substituted by 1, 2 or 3  
substituents independently selected from

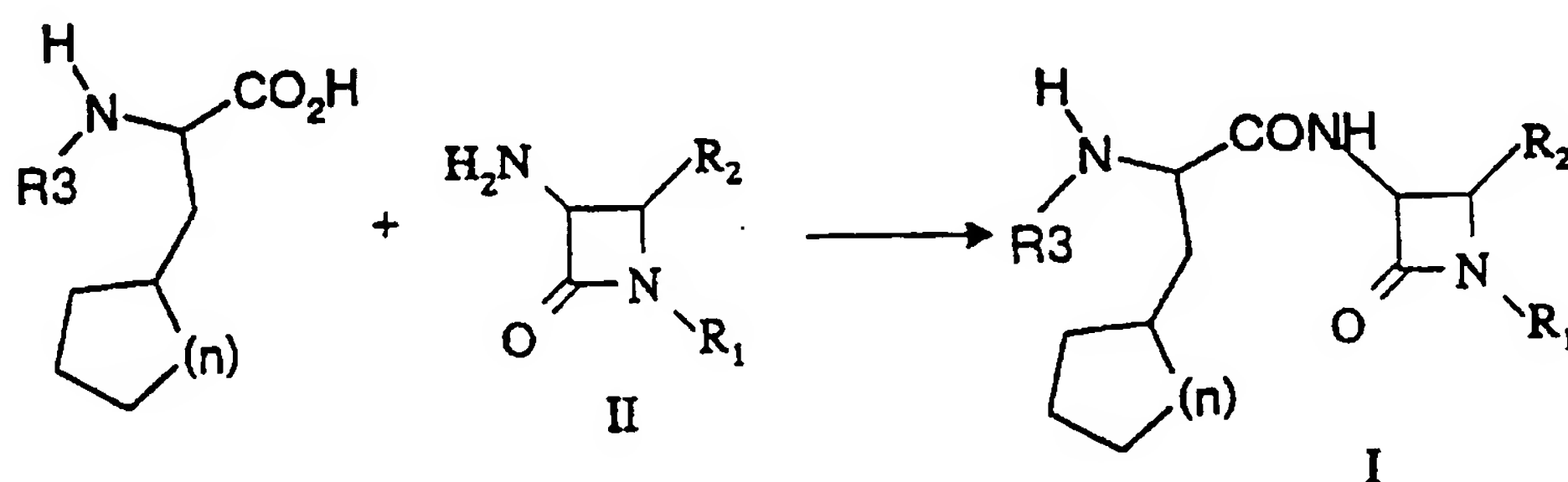
- 10 hydroxy,  
halogen,  
carboxy,  
C<sub>1</sub>-C<sub>4</sub> alkyl (which is unsubstituted or  
substituted at least once with carboxy and/or amino),  
15 C<sub>1</sub>-C<sub>2</sub> alkoxy,  
amino,  
cyano, and  
phenyl and monocyclic or bicyclic  
heterocyclic groups, which phenyl and heterocyclic groups are unsubstituted  
20 or substituted by 1 or 2 substituents independently selected from  
hydroxy,  
halogen,  
carboxy,  
C<sub>1</sub>-C<sub>4</sub> alkyl,  
25 C<sub>1</sub>-C<sub>2</sub> alkoxy,  
amino, and  
cyano;

or (b) a group -XR<sub>5</sub> wherein X is selected from the group  
consisting of O, S, SO, and SO<sub>2</sub>, and R<sub>5</sub> is as defined above;

- 30 R<sub>3</sub> is hydrogen, -COOR<sub>5</sub>, -COR<sub>5</sub>, -SO<sub>2</sub>R<sub>5</sub>, or -COR<sub>14</sub> wherein R<sub>5</sub> is as  
defined above and R<sub>14</sub> is amino group which is unsubstituted or substituted  
at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at

least once with 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle, and phenyl (wherein the heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino).

- 5 34. A method of preparing a compound of formula I, comprising reacting a compound according to formula II with a substituted cycloalkyl alanine in the presence of at least one member selected from the group consisting of dicyclohexylcarbodiimide, acid chloride, base and activated ester:



10 wherein

n is 1, 2 or 3

R<sub>1</sub> is

hydrogen;

15 -SO<sub>3</sub><sup>-</sup>M<sup>+</sup> wherein M is a hydrogen atom, a metal ion which is selected from sodium, potassium, magnesium, or calcium, or N<sup>+</sup>(R<sub>4</sub>)<sub>4</sub> wherein R<sub>4</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group;

R<sub>2</sub> is

-OCOR<sub>5</sub> wherein R<sub>5</sub> is

20 (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, and amino; or

(ii) a phenyl group which is unsubstituted or substituted at least once by 1-3 substituents selected from hydroxy, halogen, C<sub>1</sub>-C<sub>4</sub> alkyl group, C<sub>1</sub>-C<sub>2</sub> alkoxy group, and cyano;

25

-XR<sub>6</sub> wherein X is O, S, SO, or SO<sub>2</sub>; R<sub>6</sub> is

(i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or

substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, amino or phenyl; or

- (ii) a phenyl group which is unsubstituted or substituted at least once by 1-3 substituents selected from hydroxy, halogen, carboxy, and C<sub>1</sub>-C<sub>4</sub> alkyl group which is unsubstituted or substituted at least once with carboxy, amino or both, C<sub>1</sub>-C<sub>2</sub> alkoxy group, cyano or heterocycle group;

R<sub>3</sub> is

hydrogen;

- COOR<sub>7</sub> wherein R<sub>7</sub> is a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with phenyl and/or heterocycle group;

-COR<sub>8</sub> wherein R<sub>8</sub> is

- (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle, and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino; or

- (ii) an amino group which is unsubstituted or substituted at least once with C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once by 1 or 2 substituents selected from hydroxy, halogen, cyano, amino, heterocycle and phenyl, wherein said heterocycle or phenyl is unsubstituted or substituted at least once by 1 or 2 substituents selected from halogen, hydroxy, cyano, carboxy and amino; or

-SO<sub>2</sub>R<sub>9</sub> wherein R<sub>9</sub> is

- (i) a C<sub>1</sub>-C<sub>6</sub> alkyl group which is unsubstituted or substituted at least once with heterocycle and/or phenyl; or

- (ii) a C<sub>2</sub>-C<sub>4</sub> alkenyl group which is unsubstituted or substituted at least once with heterocycle and/or phenyl.

35. A compound selected from the group consisting of:

- (3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexyl - methyl-acetamido)-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -

hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl) -  
phenoxy}-azetidin-2-one;

5 (3S,4R)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-{4-(2S-2-amino-2-carboxy-ethyl) -  
phenoxy}-azetidin-2-one;

(3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-phenylthio-azetidin-2-one;

10 (3S,4SR)-3-{2S-2-(3-phenylpropionoyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-phenylsulfonyl-azetidin-2-one;

(3S,4S)-3-{2S-2-(benzylaminocarbonyl)amino-2-cyclo -  
hexylmethyl-acetamido}-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{2S-2-(phenylethenylsulfonyl)amino-2-  
15 cyclohexylmethyl-acetamido}-4-acetoxy-azetidin-2-one;

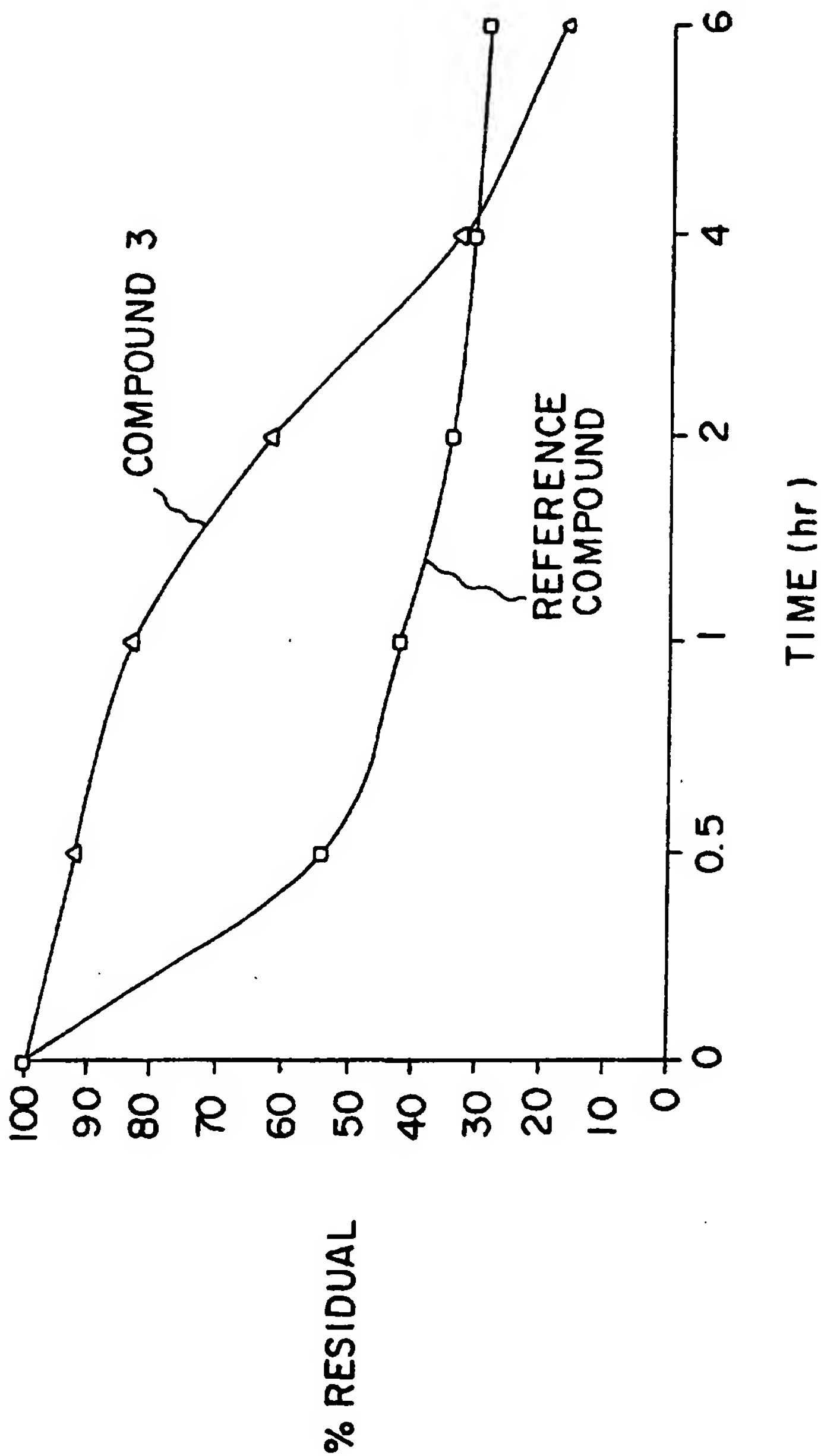
(3S,4S)-3-(2S-2-benzyloxycarbonylamino-2-cyclohexylmethyl -  
acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one;

(3S,4R)-3-(2S-2-benzyloxycarbonyl amino-2-cyclohexylmethyl-  
acetamido)-4-(3-methyl-phenoxy)-azetidin-2-one;

20 (3S,4S)-3-{2S-2-[3-(pyridin-4-yl) propenoyl]amino-2-cyclohexylmethyl-  
acetamido}-4-phenoxy-azetidin-2-one; and

(3S,4S)-3-{2S-2-[3-(pyridin-3-yl) propenoyl]amino-2-cyclohexylmethyl-  
acetamido}-4-phenoxy-azetidin-2-one.

1/1





# INTERNATIONAL SEARCH REPORT

Interr. Nat Application No  
PCT/IB 97/01144

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07K5/06 A61K38/55

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07K C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 393 457 A (SUNTORY LIMITED) 24 October 1990 see claims	1-35
P,A	--- WO 96 32408 A (SYNPHAR LABORATORIES, INC.) 17 October 1996 see claims -----	1-35

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

11 December 1997

Date of mailing of the international search report

18.12.97

Name and mailing address of the ISA

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Authorized officer

Chouly, J

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 97/01144

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 17-32  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 97/01144

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 393457 A	24-10-90	JP 2268145 A	01-11-90
		AT 108187 T	15-07-94
		DE 69010375 D	11-08-94
		DE 69010375 T	15-12-94
		ES 2058653 T	01-11-94
		US 5510531 A	23-04-96
		US 5081284 A	14-01-92
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